Mass Rearing *Pseudoscymnus tsugae,* a Predator of Hemlock Woolly Adelgid, at the New Jersey Department of Agriculture: Challenges and Lessons

Daniel J. Palmer[i], Alampi Beneficial Insect Laboratory, NJ Department of Agriculture, P.O. Box 330, Trenton, New Jersey 08625, and Jennifer L. Sheppard, Alampi Laboratory, NJDA.

Abstract

In developing a mass production program for the hemlock woolly adelgid (*Adelges tsugae* Annand) predator *Pseudoscymnus tsugae* Sasaji & McClure, the New Jersey Department of Agriculture learned that biological factors of the adelgid affect successful reproduction of the predator. To interpret the predator's population trends, it is essential to take into account the quality of the adelgid on which the predators have been feeding. Factors of adelgid quality include the proportion of woolly masses produced in the current and in the proceeding seasons, the population density of woolly masses on the branches, the generation and stage of development of the adelgid, and the viability and vigor of the adelgid population. Laboratory experience also has suggested there may be other, yet-unidentified adelgid factors, perhaps associated with the stress history of host trees, responsible for reproduction trends in the predator population.

Introduction

The Alampi Beneficial Insect Laboratory at the New Jersey Department of Agriculture is an insect rearing facility with a long history of developing and performing mass production programs for beneficial insects. Because of its rearing experience and the Department's previous work with hemlock woolly adelgid, the USDA Forest Service in 1997 asked the NJDA to establish a laboratory colony of *Pseudoscymnus tsugae* as a safe-guard for the insect material that then existed solely at the Connecticut Agricultural Experiment Station. The Forest Service also asked New Jersey to develop a mass production procedure for *P. tsugae* to provide a supply of the adelgid predator for distribution to states and workers interested in investigating the biological control of hemlock adelgid.

In spring 1997, Dr. Mark McClure, Connecticut Agriculture Experiment Station, gave the Alampi Laboratory 100 beetles to start work. From that beginning, NJDA succeeded in establishing a colony of its own and in devising a production method that in four rearing seasons produced 450,000 *Pseudoscymnus* for distribution to ten states, two Federal recreation areas, and numerous researchers for release or study.

Having received 100 *Pseudoscymnus* and facing a universe of choices of how to begin mass production, the Laboratory returned to a familiar rearing scheme of using gallon glass jars as oviposition containers and large plastic boxes for development of the eggs.   Female beetles held in jars lay eggs on bouquets of adelgid-infested hemlock. After a week, the egg-laden bouquets are transferred to plexiglas boxes with masses of infested hemlock for development of the hatching predator larvae. Ultimately a new generation of beetles emerges, is collected by gentle aspiration, and is stored or released as the season allows. The process is repeated weekly on a grand scale throughout the production season.

Although the procedure is simple, the devil is very much in the details: very small errors in rearing conditions, handling, or feeding have very large consequences in reducing production returns. Many problems had to be recognized and solved.

The rearing of *P. tsugae* is still evolving at the NJDA, but the work thus far has presented stimulating challenges and revealing lessons.

A Challenge: How to Measure Production

Because *P. tsugae* lays eggs singly, hiding them at the base of the needle petiole or in bark crevices, a way had to be devised to count eggs, if we were to have a reliable measure of production and if we were to recognize successes and problems as the rearing process developed. Having been successful in the past in inducing other insects to lay eggs on artificial substrates, we offered the beetles a spectrum of materials on which to oviposit. Although a favorite of other coccinellids, plastic in different forms failed; paper of different weights and textures received some eggs, but not a sufficient number to begin mass production; cotton gauze of the type used in first aid, however, if laid on an infested hemlock twig, was found to be almost as satisfactory an oviposition site as the hemlock itself.

We then began to lay squares of gauze on the hemlock bouquets in the oviposition jars. We not only found the beetles laid predictable numbers of eggs on the gauze, but that the numbers of eggs on gauze and twigs were approximately equal. Doubling the week's count of gauze eggs for all oviposition jars gave us a weekly index of egg production throughout the season. By counting those gauze eggs added to a plastic development box and comparing that number to the newly emerged adults recovered from the box several weeks later, we calculated percent successful development of the eggs. Both numbers, egg production and the percent successful development of eggs, are critical parameters in mass production. Having found that *Pseudoscymnus* could be induced to lay eggs in a place were we could count them, we now had a way to measure and direct the development of the rearing process. We could compare predator production at different times or under different circumstances, and we could identify problems or successes as they occurred.

Another Challenge: Recovering Predators from Rearing Boxes

Increased *Pseudoscymnus* production quickly presented another challenge: how could newly emerged beetle adults be collected from the masses of hemlock in development boxes?

In the presence of hemlock adelgid, *Pseudoscymnus* adults and larvae rest on hemlock branches or inconspicuously search them for food. The beetles do not readily take flight and light gradients, alternate food sources, or vigorous shaking of the plants were little help in attracting the beetles away from the hemlock for easy collection. Initially, to recover the new beetle adults from development boxes, technicians searched hemlock twig by twig. The man-hour cost to collect even a few hundred beetles this way was very high, a clear obstacle to increasing the Laboratory's production capacity.

An alert technician, however, noticed that when development boxes were moved from 55°F to 78°F, beetles hiding in hemlock filling the lower half of the box would fly to the ceiling of the box where they could be quickly collected with a small aspirator. As numbers of beetles were collected from the top of the box, additional beetles would move out of the hemlock over several hours to take their place. This flushing technique allowed one technician to collect thousands of *Pseudoscymnus* in the same time it previously took many technicians to collect several hundred.

Effects of the Host on the Reproduction of the Predator: A Critical Lesson

As the rearing process developed and we compared production returns, we recognized that the rate of successful reproduction of the predator varied according to certain biological characteristics of the host. It became clear that there can be great differences in the hemlock adelgid at different times or from different places and that the beetle's reproductive performance is a very sensitive barometer of those differences. How many eggs *P. tsugae* lays or how successfully those eggs develop to adults is very much associated with quality differences in the adelgid on which they feed.

We learned that adelgid quality is the interaction of several biological factors:

- The relative proportion of woolly masses from the previous season (old wool) *vs*. masses from the current season (new wool).
- The density of the adelgid population on the branch.
- Whether the adelgid is in the sistens or the progrediens generation.
- What stage of development the adelgid population has reached within the generation (*i.e.* Is the population predominantly dormant nymphs, immature adults, mature adults with eggs and crawlers?)
- How many of the adelgids are alive? How vigorous are they?

- Yet-unidentified factors (possibly chemical, physiological) relating to the stress history of the hemlock tree.

The most important lesson we have learned is that to understand population trends of the predator it is essential to understand the quality of the adelgid population in which they are working.

Assessing the adelgid's quality is not difficult, but it requires more than a cursory view. A close, considered look at adelgids on the branch, especially if repeated over time, gives the information necessary to assess the host's quality and to form an expectation of the predator's reproductive potential in that population.

New wool *vs.* old wool

A quick look at a wool-covered hemlock branch cannot give an accurate impression of how much adelgid is available for *Pseudoscymnus* to feed on. Old wool, if present, is empty of living adelgids and, if not recognized, makes the predator's food supply seem higher than it actually is.

When looked at carefully, new woolly masses are generally quite round with a well-defined edge. The wool is densely matted, usually pristine white, with no particulate material imbedded. Old wool is diffuse, often spread in an amorphous form along the branch, with particles of cast skins and dirt among the woolly fibers. When compared to new wool, old wool often has a gray, weathered appearance. New wool can appear on either the present season's growth or the old growth of the branch; old wool, because it remains from the previous season, can only appear on previous seasons' growth.

New and old wool are often mixed together. It is important to distinguish between the two for an accurate understanding of how many living adelgids are available for the predator to reproduce on.

Host density

Host density is the easiest factor of adelgid quality to assess. In the laboratory, reproduction of *Pseudoscymnus* is highest when offered hemlock with 15 or more new woolly masses per 2 cm of branch length. Production returns in the laboratory diminish as the number of woolly masses declines.

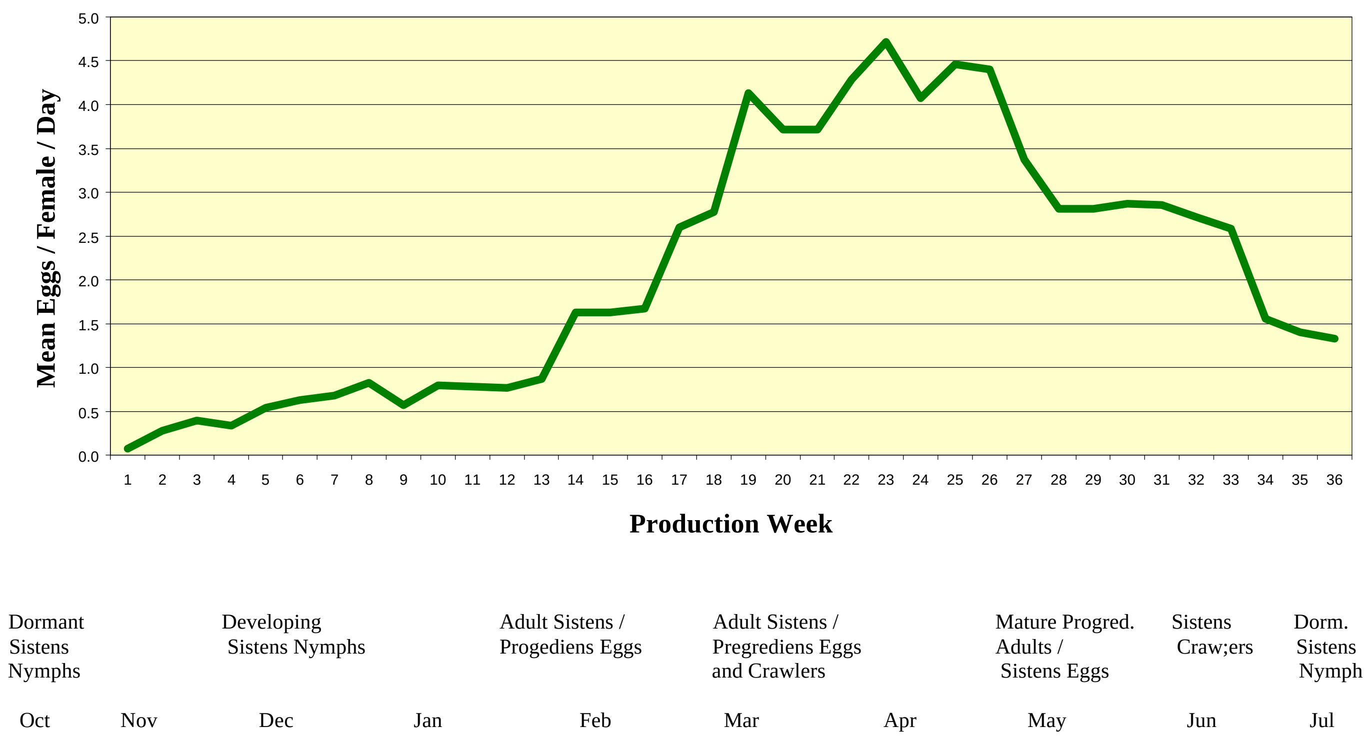
We do not suggest that actual host density levels preferred in the laboratory indicate host densities necessary to establish *Pseudoscymnus* populations in the field. Such data are presently not available. Our experience does tell us, however, that when all other quality factors are equal, predator numbers build more rapidly as host density increases.

Adelgid development: generation and stage

The predator's reproduction varies with the generation of the adelgid and stage of development within the generation. *Pseudoscymnus* have not reproduced in the laboratory when the adelgid is available only as dormant sistens nymphs. Adult beetles lay few, if any, eggs when feeding on dormant adelgids, and *Pseudoscymnus* larvae are cannibalized or die when only dormant hosts are available.

As the adelgid emerges from dormancy in October and November, the beetle lays increasingly more eggs, reaching a maximum for the season in April and May when progrediens eggs and crawlers are abundant. Eggs produced per female beetle decline as sistens eggs predominate in June and as sistens crawlers progress to their dormant stage in July (Figure 1).

Figure 1. *P. tsugae* Laboratory Egg Production 1998



Host viability

P. tsugae reproduces well only on viable hosts with good vigor. But viability and vigor of adelgids within the woolly masses are difficult factors of quality to evaluate. Healthy adelgids have a clear, colorless droplet of metabolic waste inside the woolly masses and have hemolymph of a clear wine color. Observed under moderate magnification (30X) at room temperature, healthy adelgids show slow leg movements. Dead or dying adelgids, however, have no colorless metabolic droplet present in the wool, legs are motionless, and hemolymph is cloudy brown or black.

Examined under a microscope, a healthy adelgid's body wall rebounds when lightly touched with a probe; the indentation remains, in a dead or dying adelgid. Moribund individuals (whose body wall remains indented but whose legs move) sometimes recover and resume development if environmental conditions improve, but they should be considered poor quality host material as long as their unhealthy characteristics persist.

Recognizing a woolly mass as the current season's wool tells nothing about the viability of the adelgid inside. Since new masses often contain dead or dying adelgids, it is necessary to dissect a sampling of masses from the host population to determine whether the adelgids are indeed alive. Vitality of adelgids can vary greatly even in hemlock found on neighboring trees, so a representative sample is advisable.

<u>Host vigor</u>

While there is presently no quantitative measure for adelgid vigor, we have observed qualitative characteristics of trees and adelgids that have allowed a reliable prediction of how well *Pseudoscymnus* will reproduce on that host material. In the laboratory, high rates of predator production have been associated with large, rapidly developing adelgids collected from trees with abundant new growth, especially if the new growth is at least 5 cm long. Predator reproduction is often noticeably lower on hemlock showing no new growth even if the density and viability of the adelgid appear normal.

<u>Unidentified factors</u>

Rearing experiences in New Jersey suggest there may be yet-unidentified factors, perhaps associated with the trees' response to accumulating stresses, that affect the adelgids' suitability as a host for P. *tsugae*.

In 2001, many hemlock groves in New Jersey were rebounding with new growth after having been in decline. The trees had experienced a year of good moisture (2000) following several years of heavy adelgid infestation (1996 – 1998) and a severe drought (1999). Except for having a rate of development approximately 30 days slower that in previous years, the adelgids being brought from the field in 2001 to support the laboratory's predator rearing appeared be good quality and were expected to produce high numbers of *Pseudoscymnus*. Beetle production on that New Jersey material, however, was only half that of previous years. Beetles from the same rearing stock sent out-of-state to another rearing facility and fed field adelgids with a different history produced abundant predators.

The predators in New Jersey's 2001 colony laid fewer eggs, had few larvae completing development, and, instead of resting or feeding quietly, searched their rearing containers endlessly despite the presence of abundant adelgids. We also noticed generalist predators (*Harmonia axyridis, Chilochorus* spp., and *Chrysopa* spp.), customarily on the hemlock arriving from the field, were noticeably absent from New Jersey's hemlock throughout the season, though they were present on hemlock in neighboring states.

The disparity in production for beetles of the same stock fed host material from different sources, the beetles' endless searching despite the presence of abundant food, and the unaccustomed absence of generalist predators led us to conclude that the adelgids, though seemingly normal, were in some way unacceptable to the predators. Finding other identifiable rearing factors to be the same as in previous years, we concluded that yet-unidentified factors within the adelgid itself, factors perhaps associated with the stress history of the trees, may have made the adelgids unacceptable.

In 2002, feeding exclusively on host material from states with young adelgid infestations, *P. tsugae* at NJDA has returned to the high levels of mass production we had known before.

Field Implications

Throughout more than four seasons mass producing *Pseudoscymnus tsugae*, the Alampi Laboratory has never experienced one production year quite like another. Total production numbers for each year varied; the number of predator eggs laid by a uniform group of females each week and how many of those eggs completed development changed frequently. Once we learned to examine closely the characteristics of the host we were feeding the beetle colony, we could explain every shift in the predator's production by some difference in the adelgid. It became clear: when *Pseudoscymnus* production declined or exploded, the question to ask was not what has changed with the beetle, but rather what is different about the adelgid.

We strongly believe the same thinking is necessary in the field. If *Pseudoscymnus* is difficult to find in one location and abundant in another, if it is detectable at one time but not at another, if its population builds rapidly in one place and slowly somewhere else, the condition of the adelgid population must be known before the status of the predator population can be understood.

P. tsugae is synchronized with its host, is long-lived (as long as 12 months in the laboratory), consumes large numbers of adelgids, and, having multiple generations in one season, it builds populations in short periods of time. It even endures for some days in the absence of food or water (Palmer and McCay, unpublished). In short, *Pseudoscymnus tsugae* is a rugged, productive insect that offers great promise as a potential biological control for hemlock woolly adelgid. But if the sensitive relationship between host and predator is not recognized, if the effects that qualities of the adelgid have on the beetle's reproduction are not taken into account, our ability to fully evaluate *Pseudoscymnus*' potential will be impaired.

Acknowledgments

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[i] Email: daniel.palmer@ag.state.nj.us